Current Science International 3(4): 436-444, 2014
ISSN: 2077-4435

Estimation of Zooplankton Grazing Process in Fresh and Drainage Water

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ABSTRACT

Zooplankton and bacteria are important components of the food web. To estimate the effect of zooplankton in reduction the presence of faecal indicator bacteria, a 24 hour experiment was carried out to examine the grazing pressure of zooplankton on the bacterial community as main goal. The study concerned with two types of water samples, (1) Nile sample from Rosetta branch and (2) Drainage sample from El-Rahawy drain. The results revealed that the grazing pressure was different between the two samples depending on the grazer type. Statistical analysis shows that the main grazer in Nile sample were *Keratell sp.* (r= -0.69) and *Difflugia sp.* (r= -0.62 and – 0.52), while in El-Rahawy sample the main grazer was *Phillodina sp. (*r= - 0.91) and *Vorticella sp.* (r= - 0.5).The grazing experiment confirmed the positive effect of protozoa and rotifers in the reduction of bacterial abundance. In conclusion, zooplankton can play a very important role in biological control against bacterial indicators, suggesting using this natural relation in the pre-treatment of drainage water to eliminate faecal bacterial pollution of El-Rahawy drain.

Key words: Bacteria, zooplankton, grazing, Rosetta branch, El-Rahawy drain

Introduction

Bacteria and zooplankton are important components of the food web and major contributors to biodiversity and biogeochemical processes. Although both inhabit the same environment, they are often treated as separate functional units only indirectly connected via nutrient cycling and trophic cascades (Azam and Malfatti 2007).

Zooplankton organisms are identified as important components of aquatic ecosystems. They help in regulating algal and microbial productivity through grazing and in the transfer of primary productivity to fish and other consumers (Dejen *et al.,* 2004; Nehad and Shawky, 2012).

Owing to the important ecological role of zooplankton in energy and matter transfer in food webs, its grazing studies are of great importance. Much attention has been paid to grazing on bacterioplankton in freshwater ecosystems. Although in natural environments many of zooplankton species feed on both bacteria and algae (Ooms-Wilms, 1997), grazing experiments have mostly been conducted on a single food object, and relatively little information is still available about the simultaneous grazing of zooplankton on phytoplankton and bacteria (Kim *et al*., 2000; Nehad and Shawky, 2013).

Bacteria constitute an important food resource especially for protozoan, rotifer, and crustacean (Sherr and Sherr 2001, Yoshida *et al*., 2001). The growth rate of bacteria is known to be one of the fastest among unicellular organisms, if suitable conditions prevail. Despite such characteristics, however, in natural ecosystems, the bacterial abundance does not vary drastically but remain stable within a limited magnitude in a given ecosystem (Gurung, *et al.* 2002).The higher grazing rate by microbial grazers implies the substantial role of material flux by bacterial production. Both laboratory and field studies have shown that grazing as well as resource supplies are important factors determining bacterial dynamics in aquatic ecosystems (Burns and Galbraith, 2007).

Several authors (Vaque and Pace, 1992; Ooms-Wilms, *et al*., 1995) concluded that bacterial standing crop can be suppressed by zooplankton grazing, i.e. that the microbial loop can experience top-down control, as occurs in the grazing food chain. The impact of protistan grazing on bacterial communities is based on the complex interplay of several parameters. These include grazing selectivity (by size and other features), differences in sensitivity of bacterial species to grazing, differences in responses of single bacterial populations to grazing (size and physiology), as well as the direct and indirect influence of grazing on bacterial growth conditions (substrate supply) and bacterial competition (elimination of competitors) (Martin and Manfred 2001).The size of bacteria is an important trait that strongly influences the predation of bacteria by protists (Qinglong, *et al.* 2004).In addition, protozoan selective feeding may be influenced by prey cell size and shape (Corno and Jürgens, 2006), motility, cell surface characteristics, and biochemical composition (Shannon, *et al.* 2007; Matz, 2008; Gruber, 2009), as well as predator feeding history (Ayo, *et al.* 2009). The mechanisms by which protozoa identify and selectively feed on different prey organisms are not well understood (Montagnes, *et al.* 2008; Andrew, *et al.* 2011).

There are various assays available for measuring zooplankton grazing on bacteria: fluorescent artificial particles, fluorescent-stained bacteria, dilution method, fractionated filtration, protozoan inhibitors, and radiolabelled-bacteria (Koton-Czarnecka and Chróst, 2002).

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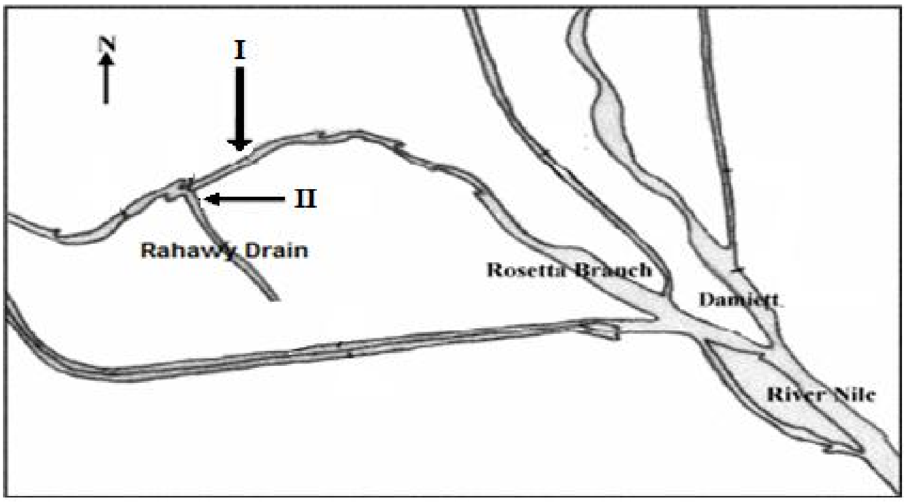
The main goal of the study is to examine the grazing pressure of zooplankton on the bacterial community. A comparison between Nile water and drainage water from El Rahawy drain regarding the grazing pressure and determination of the main grazer in both water bodies are discussed in this study.

Materials and Methods

Sampling Site

The area of investigation is 30 Km to the North of Cairo (El-Kanater El-Khayria). In this study, two different water samples were collected. The first sample collected from a control point of Rosetta branch of the river Nile one kilometer upstream El-Rahawy drain, the second sample collected from El-Rahway drain to follow its effluents effect. El-Rahawy drain is one of the main drains, which outlet into Rosetta branch of the River Nile, and receives considerable different waste waters from Greater Cairo area. There are two main sources of pollution, which potentially affect and deteriorate the water quality of El-Rahawy drain; agricultural and sewage wastes (Badr, *et al.* 2006 and El Bourie, 2008).

Twenty liters were collected with a plastic container from the subsurface layer, one meter away from the bank from each sampling site and transferred immediately to the Lab.



Sampling sites from Rosetta branch (I) and El-Rahawy drain (II).

Water temperature and pH were measured in the field at depth of 50-100 cm using pH meter (INO Lab. WTW, pH electrode Sentix 41). Samples for chemical and physical analysis were collected in specific bottles from each point and preserved in ice tanks until transported to Lab. for analysis according to (APHA, 1998).

The experiment design was to examine the numerical response of bacteria to plankton grazers at different time intervals (zero time, after 2, 4, 6, and 24 hours).

Protozoa count

Protozoa samples at each time interval were fixed by adding 1 ml of 4% formalin and concentrated to about 25 ml. One ml from the sub-samples were transferred to a Rafter Cell and examined under a compound binocular microscope.

Bacterial count

Water samples were examined at zero time, 2 hours, 4 hours, 6 hours and 24 hours intervals for heterotrophic bacterial count using nutrient agar by pour plate method, total coliforms using m-Endo agar LES medium (Difco, USA) and fecal coliforms using m-FC agar medium (Difco, USA) as membrane filter technique was applied. Results were recorded as Colony Forming Unit (cfu/100 ml) using the following equation:

Colonies / 100 ml = counted colonies/ ml of sample filtered x 100

Correlation coefficient was performed using Microsoft Excel to measure the relation between physicochemical parameters, bacterial indicators and zooplankton groups in order to verify the correlation between different variables.

Results and Discussion

Physical characteristics of investigated areas:

Results showed constant average temperature and pH in both samples. Biochemical oxygen demand (BOD) is a measure of the amount of dissolved oxygen removed from water by aerobic bacteria for their metabolic requirements during the breakdown of organic matter. The values of BOD were within the permissible limits (6 mg/l) according to the Egyptian Law 48/1982 in the Nile, but in El-Rahawy the result exceeded the Law reflecting the adverse effect of organic wastes discharge in El-Rahawy drain, which agrees with Elewa, *et al.* (2009).
Mean values of total nitrogen, ammonia, TDS, EC, orthophosphorus, and chlorophyll, recorded high levels in El-Rahawy drain compared with River Nile samples (Table 1).

Table 1: Average values of some physical and chemical parameters of the Nile and El-Rahawy samples.

	Temperature (C°)	pH	EC (µmohs)	TDS (mg/l)	BOD (mg/l)	Ammonia (mg/l)	Total nitrogen (mg/l)	Orthophosphorus (mg/l)	Chlorophyll
River Nile	30	7.65	0.35	224	5	‹ 0.2	0.916	0.52	10.73
El-Rahawy	30	7.6	1.14	734*	50*	62*	103	1.87	16.55

*Exceeding 9/2009 Egyptian Environmental Law limits: TDS≤ 500; BOD ≤ 15; Ammonia ≤ 0.5

This study was concerning with studying grazing in Rosetta branch water and El-Rahawy drain. The results of the total bacterial count (TBC) in the Nile water sample decreased gradually from zero time to the end of the experiment (24 hours). The (TBC) count started at zero time with 280 x 10^2cfu/100 ml and decreased after 2 hours to give 220 x 10^2cfu/100 ml. After 4 hours the density was190x 10^2cfu/100 ml, dropped to 95x 10^2cfu/100 ml after 6 hours. By the end of the experiment (after 24 hours) the total bacterial count recorded 70x 10^2cfu/100 ml. The total coliform (TC) was 85 x 10^2cfu/100 ml at zero time dropped after 2 hours to give 77 x 10^2cfu/100 ml and by the end of the experiment recorded 68, 57 and 16 x 10^2cfu/100 ml after 4 hr., 6 hr. and 24 hours, respectively. In case of faecal coliform (FC), the count was quite stable starting with 40 x 10^2cfu/100 ml at zero time and 35 x 10^2cfu/100 ml after 2 hours. After 4, 6 and 24 hours recorded26, 20 and 6 x 10^2cfu/100 ml, respectively (Fig. 1).

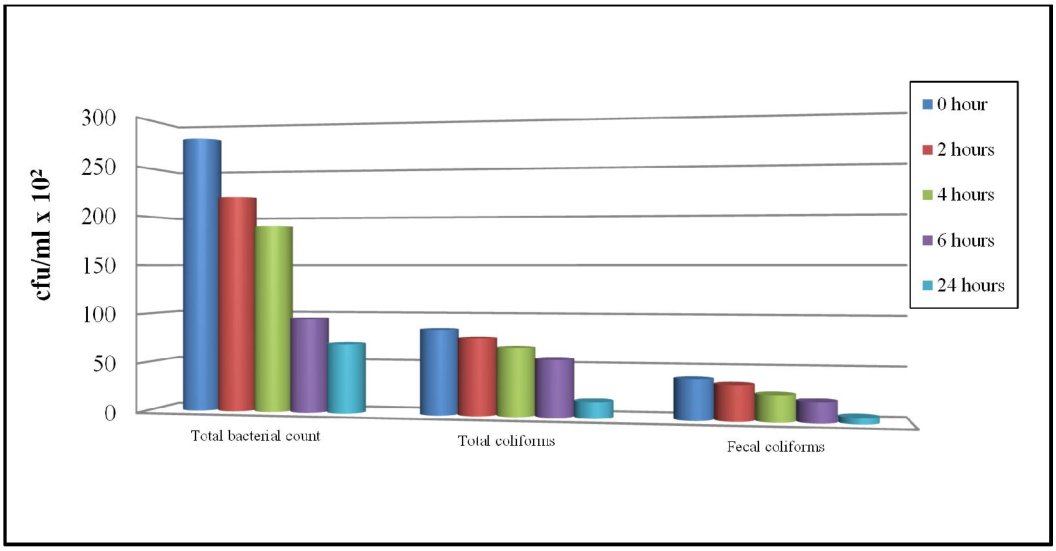


Fig. 1: The bacterial count of Nile water sample

In case of El–Rahawy sample, the total bacterial count (TBC) started with 283 x 10^5cfu/100 ml decreased to 202 x 10^5cfu/100 ml, 180 x 10^5cfu/100 ml,163 x 10^5cfu/100ml and dropped to 126 x 10^5cfu/100ml after 2, 4, 6 and 24 hour, respectively. The total coliform (TC) recorded 125 x 10^5cfu/100 ml at zero time and 107 x 10^5 cfu/100 ml after 2 hours, then decline after 4 hours to give 88 x 10^5cfu/100 ml and after 6 giving 50 x 10^5cfu/100 ml and 24 hours recorded 21 x 10^5 cfu/100 ml.

The faecal coliform (FC) recorded 25 x 10^5cfu/100 ml, 16 x 10^5cfu/100 ml, 10 x 10^5cfu/100 ml, 6 x 10^5cfu/100 ml and 2 x 10^5cfu/100ml at zero, 2, 4, 6 and after 24 hours, respectively (Fig. 2).
Results showed that count of different types of bacteria started to decrease gradually due to grazing stress in both Rosetta and El-Rahawy samples from zero time until the end of the experiment after 24 hours (Table 2 & Fig. 3).

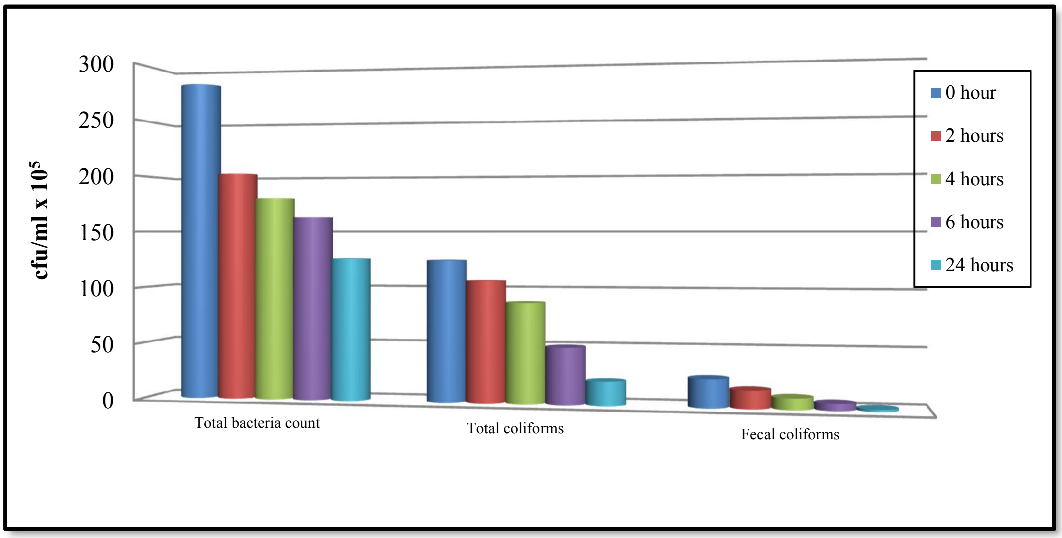


Fig. 2: The bacterial count of El-Rahawy drain

Table 2: Reduction rate percentage in bacterial count.

	After 2 hr.		After 4 hr.		After 6 hr.		After 24 hr.	
	N	W	N	W	N	W	N	W
TBC (%)	21.4	28.6	32.1	36.4	66.1	42.4	75	55.5
TC (%)	9.4	14.4	20	29.6	32.9	60	81.2	83.2
FC (%)	12.5	36	35	60	50	76	85	92

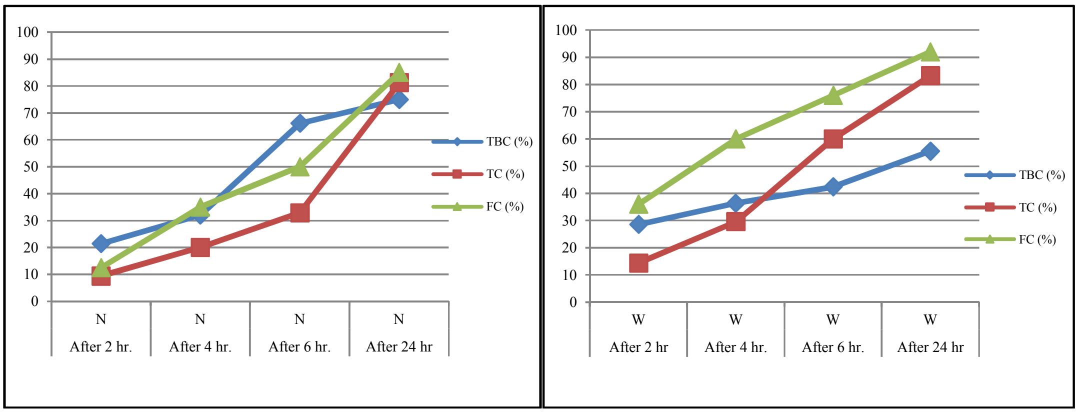


Fig. 3. Reduction percentage of bacterial count during the grazing experiment.

Ministry of Health, Egypt (1996) accepted the guide values of the investigated bacteria up to 500/100 ml water for TC and 100/100ml water for FC in freshwater. Thus, the indicator bacteria exceeded the acceptable level at the two sites, which revealed that Rosetta Branch and El-Rahawy drain waters subjected to sewage pollution.

In freshwater ecosystems, rotifers are more abundant than other zooplankton groups; therefore, they account for a major portion of the food chain (Dirican *et al*., 2009). Rotifers represent one of the most important components of the freshwater zooplankton especially in organically polluted (eutrophic) areas (Aboul-Ezz *et al*. 1996).

The study showed the inhibitory effects of sewage and industrial effluent on zooplankton diversity; the lowest species number at the discharged point of drain effluent. The same phenomenon was recorded by Ahmed (2000) and Mostafa (2002).In addition, the results showed that rotifers and protozoa were the two main zooplankton grazers in the experiment.

The rotifers were represented in both Nile water and El-Rahawy with 9 genera: *Trichocerca, Polyarthra, Anaeropsis, Conochilus, Synchyte, Philodina, Keratella, Lycane* and *Brachinus*. The most dominant species were *Trichocerca and Anaeropsis* in Nile water sample. In case of El–Rahawy sample the dominant species were *Conochilus* and *Lycane* (Fig. 4).

In case of Protozoa, it was represented with 6 genera: *Vorticella, Strombidium, Burselosis, Tintinnidium, Difflugia* and *Arcella*. The most dominant species were *Voricella* and *Difflugia* (Fig. 5).

Curr. Sci. Int. 3(4): 436-444, 2014

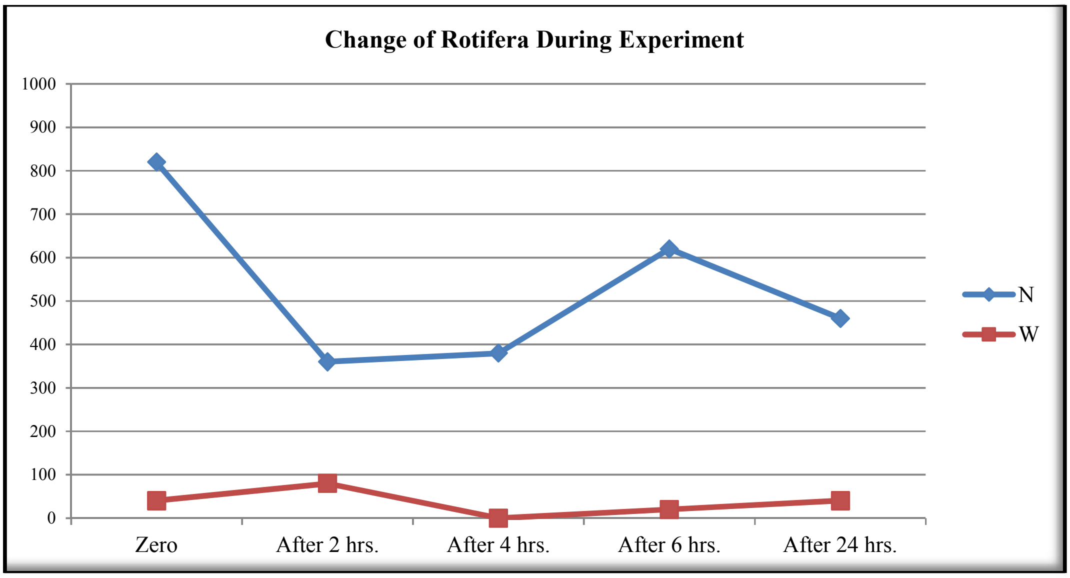


Fig. 4: Rotifers count change during the experiment.

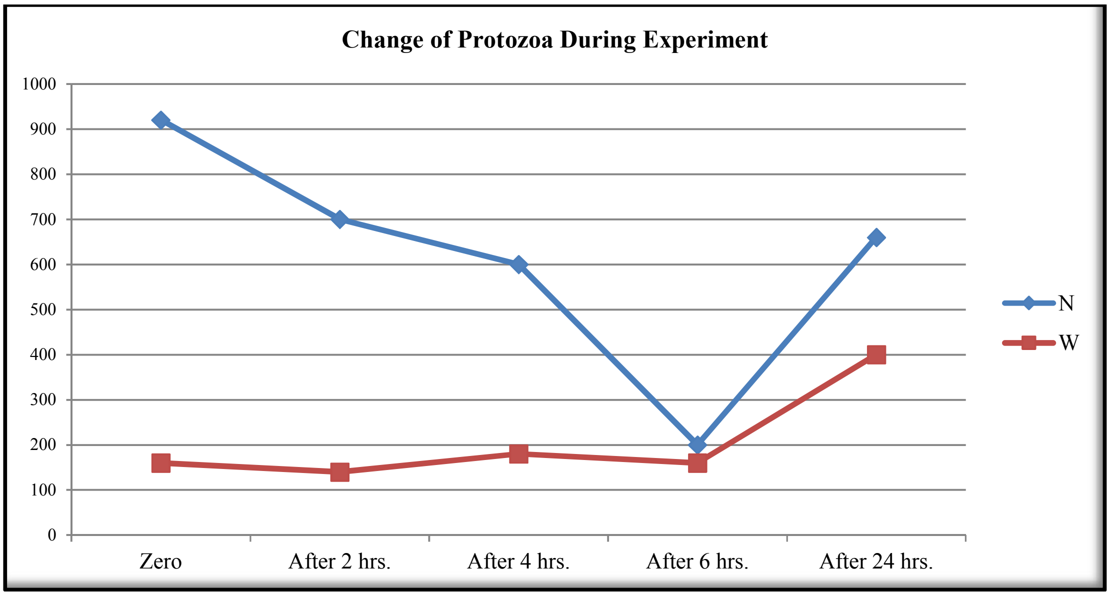


Fig. 5: Protozoa count change during the experiment.

At zero time rotifer constituted 47.1% of total zooplankton, while protozoa formed 52.9 % of total zooplankton crop in Nile water. In El-Rahawy drain rotifer constituted 20 % and protozoa was 80 % at zero time. After 24 hours, rotifers formed 41.1 % and protozoa 58.9 % of zooplankton crop of Nile water, while in El-Rahawy drain it was 9.1 % and 90.9 % for rotifers and protozoa, respectively.

During the period of experiment, rotifers showed a peak in Nile water after 6 hours forming 75.6 %, while protozoa showed three peaks at 2, 4 and after 24 hours forming 66 %, 61.2 % and 58.9 %. In case of El-Rahawy drain sample, rotifers had one peak after 2 hours with 36.3 % and protozoa was represented 100 % after 4 hours and other three peaks of zooplankton crop (Table 3) and (Fig. 6).

By the end of the experiment the relation between zooplankton grazers and faecal bacterial was obviously clear (Table 4).

Table 3: Total zooplankton crop during the experimental study

	Zero		2 hr.		4 hr.		6 hr.		24 hr.	
	N	W	N	W	N	W	N	W	N	W
% of Protozoa	52.9	80	66	63.7	61.2	100	24.4	88.9	58.9	90.9
% of Rotifer	47.1	20	34	36.3	38.8	0	75.6	11.1	41.1	9.1

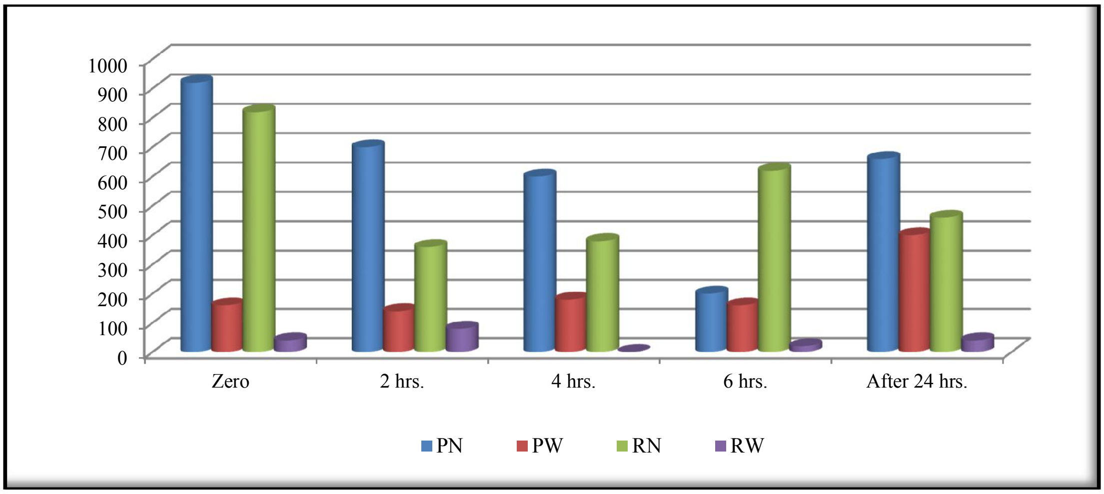


Figure. 6: Total zooplankton crop during the experimental study
N = Nile W = El-Rahawy PN = Protozoa in Nile PW = Protozoa in El-Rahawy drain
RN = Rotifer in Nile RW = Rotifer in El-Rahawy drain

Table 4: Grazer and Bacterial Count during the Experimental Study.

		Nile					El-Rahawy				
		Zero	2 hr.	4 hr.	6 hr.	24 hr.	Zero	2 hr.	4 hr.	6 hr.	24 hr.
Grazer (org./l)	Protozoa	920	700	600	200	660	160	140	180	160	400
	Rotifer	820	360	380	620	460	40	80	0	20	40
	Zooplankton	1740	1060	980	820	1120	200	220	180	180	440
Bacteria (cfu/100 ml)	TBC	280×10^2	220×10^2	190×10^2	95×10^2	70×10^2	283×10^5	202×10^5	180×10^5	163×10^5	126×10^5
	TC	85×10^2	77×10^2	68×10^2	57×10^2	16×10^2	125×10^5	107×10^5	88×10^5	50×10^5	21×10^5
	FC	40×10^2	35×10^2	26×10^2	20×10^2	6×10^2	25×10^5	16×10^5	10×10^5	6×10^5	2×10^5

Zooplankton represents the link between primary producers and secondary consumer, so it significantly influencing the food web structure (Marazzo and Valentin 2001). Zooplankton occurrence, distribution and abundance are of extreme importance in aquatic systems since they are sensitive to disturbances including eutrophication due to anthropogenic impacts such as heavy urbanization, domestic, and industrial pollutants and sewage disposal which can alter ecosystem components (Vidjak *et al.* 2009).

Protozoans play an important role as bacterial predators, acting as key components in energy flow. Predation by protozoa (top-down control) is considered to be one of the main bacterial community modifying and regulatory factors in aquatic ecosystems (Corno *et al.*, 2009).

From the result of the grazing experiment, there is almost gradual decrease of the count of different types of bacteria due to the grazing of zooplankton. These results agree with Amer (2007) and El-Bassat (2011).In addition, it was clear that zooplankton especially protozoa played a very important role in grazing bacteria followed by rotifer.

Statistical analysis

The correlation analysis for zooplankton including protozoan and rotiferan genera versus the different bacterial groups (TBC, TC and FC) in Nile water and El-Rahawy drain water revealed the following:

In the Nile sample, results revealed a highly significant negative relationship (r = - 0.987 and – 0.925, P < 0.001) for TC and FC against time, respectively.

In case of rotifer genera, *Trichocerca* and *Brachinus* were the most affecting genera during the experiment. The two genera attained a grazing pressure on the TBC more than TC and FC with relation (r = 0.659, r= 0.696.Other genera like *Keratella* showed a negative relation on TBC (r= – 0.69) reflecting low grazing pressure.

In case of protozoa, *Vorticella* was the most effecting grazing pressure on the count of different bacterial groups during the experiment. *Vorticella sp.* attained a grazing pressure on the TBC, TC and FC bacteria with relation (r = 0.75, r = 0.67, r = 0.78, P<0.01). *Difflugia* showed inverse relation on TC (r= -0.62) and FC (r= -0.52) (Table 5).

In El-Rahawy sample, the experiment duration attained a highly significant inverse relationship with the TC bacterial count (r= -0.88, P < 0.001). Among the rotifer genera, *Philodina sp.* was the most affecting species on the count of TC during the experiment. *Philodina sp.* attained a higher grazing pressure on the TC bacteria with significant indirect relation (r= -0.91) more than on TBC and FC (r= - 0.72, r= -0.79 respectively) (P<0.001). *Trichocerca sp.* was the second affecting genera only on TBC with significant positive relation (r= 0.88).

In case of protozoa, *Vorticella sp.* attained a weak negative relation (r= -0.5) on TC, while *Arcella sp.* attained a weak positive relation on different types of bacteria, TBC (r= 0.51), TC (r= 0.54) and FC (r= 0.46) (P < 0.01) (Table 6).

From the results of the grazing experiment it was clear that in the Nile sample, the protozoa have grazing pressure and responsible of bacteria count reduction. These agree with El-Bassat (2011) who reported that protozoa played a very important role in grazing bacteria followed by rotifer which had a negligible grazing potential on bacteria.

In Contrast in El-Rahawy sample, rotifer was the main grazer and has a grazing pressure effect on the bacterial community. These agree with Khalifa and Sabae (2012) who found a strong negative correlation (r= – 0.964) between total bacterial counts and rotifers density at Damietta Branch.

Conclusions

El-Rahawy drain is mainly sewage in nature and mixed with agricultural wastes. The discharge of this untreated wastewater to Rosetta Nile branch could have an adverse impact on its water quality as well as on the communities of zooplankton. The present study shows that sewage discharge affects diversity and abundance of zooplankton. In addition, grazing pressure was not the same in Rosetta Branch and in El-Rahawy drain depending on type of grazer. Rotifers (*Phillodina sp.*) and protozoa (*Vorticella sp.*) as grazers can be used for reduction of bacterial count in polluted water with sewage effluent.

In conclusion, this study suggests using this natural relation between different zooplankton genera and bacteria in the pre-treatment of drainage water to eliminate bacteria as a kind of biological control against faecal bacterial pollution of El-Rahawy drain.

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